Intensive Care Med (2008) 34:1357–1359
DOI 10.1007/s00134-008-1049-0

EDITORIAL

Jérôme Pugin

Profiling genes in the ICU: Are we there yet?

Received: 1 February 2008
Accepted: 1 February 2008
Published online: 5 April 2008

This editorial refers to the article available at: http://dx.doi.org/10.1007/s00134-008-1048-1.

J. Pugin (✉)
University Hospitals of Geneva, Department of Intensive Care, Geneva 14, 1211 Geneva, Switzerland
e-mail: jerome.pugin@medecine.unige.ch
Tel.: +41-22-3729236

Linkage disequilibrium? Synonymous SNPs? Single haplotype? Synergistic epistasis? Hierarchisation clustering? Allelic recombination? Do those terms mean anything to you? If not, you had better learn the (genetic) language (quickly)! Come on, it is not more complicated than cytokine networks, or diagrams showing intracellular signaling pathways... These terms are invading our journals dedicated to intensive care in so-called genomics studies [1]. Systems biology is another new term for a field integrating genomics, proteomics and bioinformatics.

It is important to differentiate between various types of genetic studies. Genetic studies can be done without the use of gene sequencing, and can be based on mendelian genetics, investigations in families with a particular phenotype, or in homozygous twins. Genomic studies usually have to do with mapping a trait, a small variation in the sequence of our genome (polymorphism), identifying groups of individuals who share a given polymorphism, predisposing for diseases or for a particular outcome when the disease occurs. Even before the human genome had been deciphered in 2003, it became evident that small variations existed in the genetic code between individuals. These polymorphisms correspond, for example, to a single nucleotide modification in the coding or non-coding region of a gene (single nucleotide polymorphisms, SNPs); others are insertions or deletions of a few nucleotides or repeated sequences. While most of these variations remain "silent", some of them have a significant effect on levels of protein expression and/or protein function. Human polymorphism databases now exist and are fed daily by new knowledge on those small variations linked to diseases [2]. For genomic studies to be insightful they usually need to be performed on a very large number of subjects and patients (of the same ethnic descent). Only a powerful statistical analysis is able to link genetic variations and the appearance of a disease or an increased disease severity [3]. One of the first and classical examples of this type of study in critical care is a SNP found in the promoter region of the tumor necrosis factor (TNF)-α gene promoter region, associated with increased TNF-α production, which determines both susceptibility and severity of sepsis [4]. Pharmacogenomics is one particular aspect of genomics. It refers to the study of genetic determinants causing variability of drug responses. Cytochromes and other enzymes responsible for drug metabolism, as well as drug target proteins or carriers, are sometimes very polymorphic in a given population. This largely determines responses to therapy, and partly explains individual variability of drug responsiveness. Soon, convenient and rapid genomic assays will be available in critically ill patients guiding the clinician in his therapeutic decisions [5]. Pharmacogenomic analyses will also be incorporated in clinical trials as a means to select adequate populations of patients likely to benefit from a given drug [6].

Another exciting development of systems biology is the study of variations in the expression of genes and proteins in a population of patients or in a disease ("functional genomics"). Only a minor part of the genome is transcribed into messenger RNA (mRNA, "transcriptome"), and translated into proteins ("proteome"). The process from gene activation to protein synthesis is

complex and depends largely on transcription factors binding to promoter regions of genes. The analysis of the transcriptome is usually done using microarrays. Complementary DNA made from tissue mRNA is hybridized with oligonucleotide probes corresponding to known transcripts immobilized on a chip. Modern chips span the whole transcriptome of a species; 54,000 probes can now be immobilized on a chip allowing to interrogate the whole human transcriptome on a single chip, for example. Alternatively, arrays can be customized to fit a subset of genes of interest (e.g. cancer and immunity). Levels of transcription are usually compared between two conditions (normal vs. disease, early vs. late, survivors vs. non-survivors, etc.). Transcriptomics also offer the exciting possibility of defining gene expression signatures specific for a given disease or condition [7]. It is possible, for example, to differentiate SIRS from sepsis using leukocyte gene expression profiles [8, 9], as well as the family of bacteria responsible for sepsis [10], and soon will be possible with the bacteria itself [11]. In other words, a patient will soon be identified *at the time of admission* as having a Gram-negative sepsis due to *E.coli*, based on a pattern (or cluster) of genes specific to sepsis and a detection of the causative microorganism by molecular techniques.

Post-transcriptional modifications, such as mRNA stabilization, also play a crucial role for protein expression. Cell mRNA levels, and hence protein production, depend on both the level of transcription and mRNA stability. The function of newly synthesized proteins is also highly dependent on post-translational modifications; these include, for example, protein phosphorylation, oxidation and addition of lipids, depending on the state of cell activation, and not on transcription per se. Although proteomic techniques have recently progressed, the detection of low levels of proteins in a cell lysate or in a given body fluid remains difficult. In addition, automatization and standardization of proteomic techniques are problematic and warrant their use in the clinical setting for the time being.

In this issue of "Intensive Care Medicine" Payen et al. describe their work in which they have sampled circulating leukocytes in a series of patients admitted for septic shock and during their recovery phase [12]. They measured levels of expression of 340 genes during the course of septic shock using a microarray dedicated to inflammation, and compared them with their respective expression at the time of admission. They found significant modifications in transcript levels of ten genes between day 0, day 7 and day 28 for survivors. Of these ten genes, they confirmed significant variations by PCR in 4 mRNAs, and by ELISA for one of these proteins. One of the mRNA identified was the invariant chain [CD74, a major histocompatibility complex (MHC) class-II-associated protein]. CD74 mRNA levels were downregulated at the time of admission compared with levels found later in the course of sepsis. CD74 mRNA levels correlated with the monocyte surface expression of the MHC class-II HLA-DR molecule in the same patients. This suggested that a decreased CD74 expression played a role in the low-monocyte HLA-DR expression observed early during sepsis.

Two transcripts significantly decreased during the course of septic shock: S100A8 and S100A12 mRNAs, encoding for calcium-binding proteins also know as calgranulin A and C, respectively. Plasma S100A8 protein levels decreased in the 14 survivors, but not in the 3 patients who died. S100A8 is physiologically concentrated in phagocytic granules from granulocytes and released at sites of inflammation. It carries pro-inflammatory effects when complexed with its S100A9 companion, most likely via an interaction with the Toll-like receptor 4/MD-2 complex and/or the receptor for advanced glycation end-products (RAGE) [13]. This mode of action is reminiscent of that of the high-mobility group box 1 (HMGB1) alarmin [14]. S100 proteins also carry prothrombotic effects and induce increased vascular permeability, two essential features of septic shock. Interestingly, mice lacking the S100A8 protein are protected against lethal septic shock [15]. S100A8, A9 and A12 proteins qualify as endogenous "alarmins" secreted by immune effector cells, signaling danger in the context of tissue suffering and inflammation [14]. Further studies on S100 proteins and alarmins are necessary to identify their possible pathogenic role in human sepsis and in diseases associated with a systemic inflammatory syndrome and multiple organ dysfunction [16].

This study has limitations. Firstly, although the number of genes studied seems significant ($n = 340$), it represents less than 1% of the human transcriptome. In spite of the fact that the chip used was well designed to study genes of interest in sepsis, it remains a possibility that important variations in unsuspected gene transcripts were missed with this analysis. Secondly, the study was performed on a limited number of patients with an abdominal source of infection as the cause for septic shock. Thirdly, gene profiling was done on a mixed cell population including monocytes, lymphocytes and immature neutrophils. The cellular source of the transcripts studied by Payen et al. [12] can therefore not be identified precisely. In addition, mature neutrophils were kept out of the analysis, and are likely to be an important source of S100 proteins. Finally, a limitation inherent to all of these types of studies is that the cut-off value used to decide whether a variation in transcript level is significant or not (usually twofold increase or decrease) is purely arbitrary. A 20% increase in transcription may represent a very significant variation for some genes, and will be overseen by classical transcriptomic analyses. Moreover, variations in mRNA levels do not always mean increased or decreased gene transcription, and do not always parallel protein production, as discussed previously.

Will genomic studies replace more classical research, i.e. the step-to-step study of one gene and the function of one protein in a given disease? It is very unlikely. Radio

has not replaced newspapers, and television has replaced neither radio nor newspapers. At some point, someone has to go to the bench and study that particular gene that has been identified in a transcriptomic experiment, and figure out in what exactly this gene is involved. Systems biology is very complementary to basic research. It represents a perfect interface, reinforces translational research, where the need for the clinical and basic research worlds to exchange ideas, concepts and visions is so important.

Genomics has already entered the clinical arena in oncology and is used daily for selecting patients for treatments, for example. Will the intensivist soon become a "gene profiler"? We are not quite there yet. We need to prepare ourselves, however, by learning the language and understanding the technology. While genomic analyses are at the door of our ICUs, in particular pharmacogenomics, it is generally accepted that transcriptomic and proteomic studies are still investigational tools. The analysis of the complete transcriptional network of immune cell activation has reached a very high level of complexity, as recently shown by Calvano et al. in volunteers injected with endotoxin [17]. One of the important messages of this paper is that researchers focused on "their gene" should broaden their views and integrate their gene into this complex network. Another crucial result from these studies was the identification of a dysregulation of leukocyte bioenergetics induced by endotoxin, which makes a lot of sense for the clinician caring for septic patients. Systems biology provides invaluable results and leads the way to the identification of new markers, new mediators, new pathways, and potentially new therapeutic targets to combat deadly syndromes such as septic shock.

References

1. Yende S, Kammerer CM, Angus DC (2006) Genetics and proteomics: deciphering gene association studies in critical illness. Crit Care 10:227
2. http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp
3. Stuber F (2003) Genomics and critical care: Do the right thing! Crit Care Med 31:1869–1870
4. Mira JP, Cariou A, Grall F, Delclaux C, Losser MR, Heshmati F, Cheval C, Monchi M, Teboul JL, Riche F, Leleu G, Arbibe L, Mignon A, Delpech M, Dhainaut JF (1999) Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. J Am Med Assoc 282:561–568
5. Freeman BD, McLeod HL (2004) Challenges of implementing pharmacogenetics in the critical care environment. Nat Rev Drug Discov 3:88–93
6. Cariou A, Chiche JD, Charpentier J, Dhainaut JF, Mira JP (2002) The era of genomics: impact on sepsis clinical trial design. Crit Care Med 30:S341–S348
7. Tang BM, McLean AS, Dawes IW, Huang SJ, Lin RC (2007) The use of gene-expression profiling to identify candidate genes in human sepsis. Am J Respir Crit Care Med 176:676–684
8. Johnson SB, Lissauer M, Bochicchio GV, Moore R, Cross AS, Scalea TM (2007) Gene expression profiles differentiate between sterile SIRS and early sepsis. Ann Surg 245:611–621
9. Chung TP, Laramie JM, Meyer DJ, Downey T, Tam LH, Ding H, Buchman TG, Karl I, Stormo GD, Hotchkiss RS, Cobb JP (2006) Molecular diagnostics in sepsis: from bedside to bench. J Am Coll Surg 203:585–598
10. Yu SL, Chen HW, Yang PC, Peck K, Tsai MH, Chen JJ, Lin FY (2004) Differential gene expression in gram-negative and gram-positive sepsis. Am J Respir Crit Care Med 169:1135–1143
11. Lehmann LE, Hunfeld KP, Emrich T, Haberhausen G, Wissing H, Hoeft A, Stuber F (2007) A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples. Med Microbiol Immunol, Nov 16 [Epub ahead of print]
12. Payen D, Lukaszewicz A-C, Belikova I, Faivre V, Gelin C, Russwurm S, Launay J-M, Sevenet N (2008) Gene profiling in human blood mononuclear cells during recovery from septic shock. Intensive Care Med DOI 10.1007/s00134-008-1048-1
13. Foell D, Wittkowski H, Vogl T, Roth J (2007) S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol 81:28–37
14. Bianchi ME (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 81:1–5
15. Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, van Zoelen MA, Nacken W, Foell D, van der Poll T, Sorg C, Roth J (2007) Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat Med 13:1042–1049
16. Pugin J (2007) Dear SIRS, the concept of "alarmins" makes a lot of sense! Intensive Care Med, Oct 17 [Epub ahead of print]
17. Calvano SE, Xiao W, Richards DR, Felciano RM, Baker HV, Cho RJ, Chen RO, Brownstein BH, Cobb JP, Tschoeke SK, Miller-Graziano C, Moldawer LL, Mindrinos MN, Davis RW, Tompkins RG, Lowry SF (2005) A network-based analysis of systemic inflammation in humans. Nature 437:1032–1037